

CLAIMS

What is claimed is:

1. An isolated nucleic acid sequence encoding a polypeptide with isoflavone synthase activity having the amino acid sequence set forth in SEQ ID NO:66 wherein

- 5           Xaa<sub>10</sub> is Phe or Leu  
          Xaa<sub>16</sub> is Ser or Leu  
          Xaa<sub>23</sub> is Ser or Thr  
          Xaa<sub>25</sub> is Ile or Lys  
          Xaa<sub>39</sub> is Lys or Arg  
10          Xaa<sub>48</sub> is Pro or Leu  
          Xaa<sub>60</sub> is Pro or Leu  
          Xaa<sub>73</sub> is Leu or His  
          Xaa<sub>74</sub> is Ser or Tyr  
          Xaa<sub>95</sub> is Ala or Thr  
15          Xaa<sub>96</sub> is Asn or His  
          Xaa<sub>102</sub> is Asn or Ser  
          Xaa<sub>110</sub> is Ile, Val, or Thr  
          Xaa<sub>112</sub> is Arg or His  
          Xaa<sub>117</sub> is Asn or Ser  
20          Xaa<sub>118</sub> is Ser or Leu  
          Xaa<sub>121</sub> is Met or Arg  
          Xaa<sub>122</sub> is Ala or Val  
          Xaa<sub>124</sub> is Phe or Ile  
          Xaa<sub>129</sub> is Lys or Arg  
25          Xaa<sub>147</sub> is Lys or Glu  
          Xaa<sub>159</sub> is Leu or Phe  
          Xaa<sub>162</sub> is Ala or Val  
          Xaa<sub>166</sub> is Ser or Gly  
          Xaa<sub>170</sub> is Gln or Arg  
30          Xaa<sub>175</sub> is Val or Leu  
          Xaa<sub>183</sub> is Ala or Thr  
          Xaa<sub>187</sub> is Thr or Ile  
          Xaa<sub>191</sub> is Met or Val  
          Xaa<sub>209</sub> is Phe or Tyr  
35          Xaa<sub>219</sub> is Arg or Trp  
          Xaa<sub>223</sub> is Tyr or His  
          Xaa<sub>253</sub> is Gly or Glu  
          Xaa<sub>259</sub> is Lys or Glu

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Xaa<sub>263</sub> is Val or Asp  
 Xaa<sub>264</sub> is Val, Asp, or Ile  
 Xaa<sub>268</sub> is Ala or Val  
 Xaa<sub>272</sub> is Phe or Leu  
 Xaa<sub>285</sub> is Thr or Met  
 Xaa<sub>293</sub> is Glu or Asp  
 Xaa<sub>294</sub> is Thr, or Ile

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Xaa<sub>301</sub> is Phe or Leu  
 Xaa<sub>306</sub> is Thr or Ile  
 Xaa<sub>311</sub> is Val or Glu  
 Xaa<sub>312</sub> is Val or Ala  
 Xaa<sub>325</sub> is Arg or Lys  
 Xaa<sub>328</sub> is Gln or Glu  
 Xaa<sub>334</sub> is Val or Ala

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Xaa<sub>342</sub> is Arg or Ile  
 Xaa<sub>377</sub> is Thr or Ile  
 Xaa<sub>381</sub> is Glu or Gly  
 Xaa<sub>385</sub> is Tyr, His, or Cys

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Xaa<sub>387</sub> is Ile or Thr  
 Xaa<sub>393</sub> is Val or Ile  
 Xaa<sub>394</sub> is Leu or Pro  
 Xaa<sub>402</sub> is Arg or Lys  
 Xaa<sub>404</sub> is Ser or Pro  
 Xaa<sub>413</sub> is Ser or Phe

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Xaa<sub>422</sub> is Glu or Gly  
 Xaa<sub>428</sub> is Gly or Arg  
 Xaa<sub>429</sub> is Pro or Leu  
 Xaa<sub>435</sub> is Gln or Arg  
 Xaa<sub>447</sub> is Arg or Gly

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Xaa<sub>453</sub> is Asn, Ser, or Ile  
 Xaa<sub>459</sub> is Met or Thr, and  
 Xaa<sub>485</sub> is Asp or Gly.

2. An isolated polypeptide sequence of SEQ ID NO: 66 wherein

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Xaa<sub>10</sub> is Phe or Leu  
 Xaa<sub>16</sub> is Ser or Leu  
 Xaa<sub>23</sub> is Ser or Thr  
 Xaa<sub>25</sub> is Ile or Lys  
 Xaa<sub>39</sub> is Lys or Arg

Xaa<sub>48</sub> is Pro or Leu  
Xaa<sub>60</sub> is Pro or Leu  
Xaa<sub>73</sub> is Leu or His  
Xaa<sub>74</sub> is Ser or Tyr  
Xaa<sub>95</sub> is Ala or Thr  
Xaa<sub>96</sub> is Asn or His  
Xaa<sub>102</sub> is Asn or Ser  
Xaa<sub>110</sub> is Ile, Val, or Thr  
Xaa<sub>112</sub> is Arg or His  
Xaa<sub>117</sub> is Asn or Ser  
Xaa<sub>118</sub> is Ser or leu  
Xaa<sub>121</sub> is Met or Arg  
Xaa<sub>122</sub> is Ala or Val  
Xaa<sub>124</sub> is Phe or Ile  
Xaa<sub>129</sub> is Lys or Arg  
Xaa<sub>147</sub> is Lys or Glu  
Xaa<sub>159</sub> is Leu or Phe  
Xaa<sub>162</sub> is Ala or Val  
Xaa<sub>166</sub> is Ser or Gly  
Xaa<sub>170</sub> is Gln or Arg  
Xaa<sub>175</sub> is Val or Leu  
Xaa<sub>183</sub> is Ala or Thr  
Xaa<sub>187</sub> is Thr or Ile  
Xaa<sub>191</sub> is Met or Val  
Xaa<sub>209</sub> is Phe or Tyr  
Xaa<sub>219</sub> is Arg or Trp  
Xaa<sub>223</sub> is Tyr or His  
Xaa<sub>253</sub> is Gly or Glu  
Xaa<sub>259</sub> is Lys or Glu  
Xaa<sub>263</sub> is Val or Asp  
Xaa<sub>264</sub> is Val, Asp, or Ile  
Xaa<sub>268</sub> is Ala or Val  
Xaa<sub>272</sub> is Phe or Leu  
Xaa<sub>285</sub> is Thr or Met  
Xaa<sub>293</sub> is Glu or Asp  
Xaa<sub>294</sub> is Thr, or Ile  
Xaa<sub>301</sub> is Phe or Leu  
Xaa<sub>306</sub> is Thr or Ile

Xaa<sub>311</sub> is Val or Glu  
 Xaa<sub>312</sub> is Val or Ala  
 Xaa<sub>325</sub> is Arg or Lys  
 Xaa<sub>328</sub> is Gln or Glu  
 Xaa<sub>334</sub> is Val or Ala  
 Xaa<sub>342</sub> is Arg or Ile  
 Xaa<sub>377</sub> is Thr or Ile  
 Xaa<sub>381</sub> is Glu or Gly  
 Xaa<sub>385</sub> is Tyr, His, or Cys  
 Xaa<sub>387</sub> is Ile or Thr  
 Xaa<sub>393</sub> is Val or Ile  
 Xaa<sub>394</sub> is Leu or Pro  
 Xaa<sub>402</sub> is Arg or Lys  
 Xaa<sub>404</sub> is Ser or Pro  
 Xaa<sub>413</sub> is Ser or Phe  
 Xaa<sub>422</sub> is Glu or Gly  
 Xaa<sub>428</sub> is Gly or Arg  
 Xaa<sub>429</sub> is Pro or Leu  
 Xaa<sub>435</sub> is Gln or Arg  
 Xaa<sub>447</sub> is Arg or Gly  
 Xaa<sub>453</sub> is Asn, Ser, or Ile  
 Xaa<sub>459</sub> is Met or Thr, and  
 Xaa<sub>485</sub> is Asp or Gly.

3. An isolated nucleic acid sequence encoding a polypeptide with isoflavone synthase activity.

4. An isolated nucleic acid sequence encoding a polypeptide with isoflavone synthase activity wherein the nucleic acid sequence is not the nucleic acid sequence set forth in SEQ ID NO:9.

5. The isolated nucleic acid sequence of Claim 1 at least 85% identical to the nucleic acid set forth in SEQ ID NO:1.

6. The isolated nucleic acid sequence of Claim 1 at least 90% identical to the nucleic acid set forth in SEQ ID NO:1.

7. The isolated nucleic acid sequence of Claim 1 wherein the nucleic acid hybridizes to the nucleic acid set forth in SEQ ID NO:1

8. The isolated nucleic acid sequence of Claim 1 wherein the encoded polypeptide comprises an amino acid sequence that is at least 95% identical to the amino acid sequence set forth in SEQ ID NO:2.

9. The isolated nucleic acid sequence of Claim 1 selected from the group consisting of SEQ ID NOs:1, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 47, 54, 56, 58, and 60.

10. The isolated nucleic acid sequence of Claim 1 encoding the amino acid sequence set forth in a member selected from the group consisting of SEQ ID NOs:2, 10, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 48, 55, 57, 59, 61, and 66.

11. A chimeric sequence comprising the nucleic acid sequence of Claim 1 operably linked to suitable regulatory sequences.

12. A transformed host cell comprising the chimeric sequence of Claim 11.

13. The transformed host cell of Claim 12 further comprising a second chimeric sequence comprising a nucleic acid sequence encoding a polypeptide that regulates expression of at least one enzyme of the phenylpropanoid pathway.

14. The transformed host cell of Claim 13 wherein the second chimeric sequence comprises a chimera containing the maize R region between the region encoding the C1 DNA binding domain and the C1 activation domain.

15. The transformed host cell of Claim 12 wherein the host cell is a eukaryotic cell.

16. The eukaryotic cell of Claim 13 wherein the cell is a yeast cell.

17. The eukaryotic cell of Claim 15 wherein the cell is a plant cell.

18. The plant cell of Claim 17 wherein the cell is a soybean cell.

19. The plant cell of Claim 17 wherein the cell is a corn cell.

20. A plant comprising in its genome the chimeric sequence of Claim 11.

21. The plant of Claim 20 further comprising in its genome a second chimeric sequence comprising a nucleic acid sequence encoding a polypeptide that regulates expression of at least one enzyme of the phenylpropanoid pathway.

22. The plant of Claim 20 wherein the plant is a soybean plant.

23. The plant of Claim 20 wherein the plant is a corn plant.

24. A seed from the plant of Claim 20.

25. A seed from the plant of Claim 21.

26. A method of altering the level of expression of isoflavone synthase in a host cell comprising:

- (a) transforming a host cell with the chimeric sequence of Claim 11;
- (b) optionally transforming the host cell with a second chimeric sequence comprising a nucleic acid sequence encoding a polypeptide that regulates expression of at least one enzyme of the phenylpropanoid pathway; and
- (c) growing the transformed host cell produced in step (a) or step (b) under conditions that are suitable for expression of the chimeric sequence

wherein expression of the chimeric sequences result in production of altered levels of isoflavone synthase in the transformed host cell.

27. A method of increasing the amount of an isoflavonoid in a host cell comprising:

- (a) transforming a host cell with the chimeric sequence of Claim 11;
- (b) optionally transforming the host cell with a second chimeric sequence comprising a nucleic acid sequence encoding a polypeptide that regulates expression of at least one enzyme of the phenylpropanoid pathway; and
- (c) growing the transformed host cell produced in step (a) or step (b) under conditions that are suitable for expression of the chimeric sequence

wherein expression of the chimeric sequences results in production of an amount of an isoflavonoid in the transformed host cell that is greater than the amount of the isoflavonoid that is produced in a cell that is not transformed with the chimeric sequence of Claim 11.

28. The method of Claim 26 wherein the isoflavonoid is selected from the group consisting of genestein and daidzein.

29. The method of Claim 26 or Claim 27 wherein the host cell is a eukaryotic cell.

30. The method of Claim 26 or Claim 27 wherein the eukaryotic cell is a yeast cell.

31. The method of Claim 26 or Claim 27 wherein the eukaryotic cell is a plant cell.

32. The method of Claim 31 wherein the plant cell is a soybean cell.

33. The method of Claim 31 wherein the plant cell is a corn cell.

34. A method of producing a plant with increased isoflavonoid content comprising

- (a) transforming a plant cell with the chimeric sequence of Claim 11;
- (b) optionally transforming the plant cell with a second chimeric sequence comprising a nucleic acid sequence encoding a polypeptide that regulates expression of at least one enzyme of the phenylpropanoid pathway; and
- (c) growing the transformed plant cell under conditions that promote the regeneration of a whole plant from the transformed cell

wherein the transformed plant regenerated from the transformed cell produces an amount of an isoflavonoid that is greater than the amount of the isoflavonoid that is produced in a plant that is regenerated from a plant cell that is not transformed with the chimeric sequence of Claim 11.

35. The method of Claim 34 wherein the plant is a soybean plant.

36. The method of Claim 34 wherein the plant is a corn plant.

37. The transgenic plant produced by the method of Claim 34.

38. The transgenic plant of Claim 37 wherein the plant is a soybean plant.

39. The transgenic plant of Claim 37 wherein the plant is a corn plant.

40. A seed from the plant of Claim 37.

41. A method of obtaining a nucleic acid sequence encoding all or a substantial portion of the amino acid sequence encoding a plant isoflavone synthase comprising

- (a) probing a cDNA or genomic library with the nucleic acid sequence of Claim 1;
- (b) identifying a DNA clone that hybridizes with the nucleic acid sequence of Claim 1;
- (c) isolating the DNA clone identified in step (b);
- (d) sequencing the cDNA or genomic sequence that comprises the clone isolated in step (c); and
- (e) demonstrating the functional expression of isoflavone synthase mediated by the cDNA or genomic sequence sequenced in step (d)

wherein the sequenced nucleic acid sequence encodes all or a substantial portion of the amino acid sequence encoding a plant isoflavone biosynthetic enzyme.

42. A method of obtaining a nucleic acid sequence encoding all or a substantial portion of an amino acid sequence encoding a plant isoflavone synthase comprising:

- (a) synthesizing an oligonucleotide primer corresponding to a portion of the sequence set forth in a member of selected from the group consisting of SEQ ID NOs: 1, 9, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 47, 54, 56, 58, and 60;
- (b) amplifying a cDNA insert present in a cloning vector using the oligonucleotide primer of step (a) and a primer representing sequences of the cloning vector to produce an amplified nucleic acid sequence; and
- (c) demonstrating the functional expression of isoflavone synthase mediated by the amplified nucleic acid sequence produced in step (b)

wherein the amplified nucleic acid sequence encodes all or a substantial portion of an amino acid sequence encoding a plant isoflavone synthase.

43. The method of Claim 42 wherein the oligonucleotide primer is selected from the group consisting of SEQ ID NOs: 5, 6, 7, 8, 11, 12, 13, 14, 41, 42, 49, 50, and 51.

44. The product of the method of Claim 41.

45. The product of the method of Claim 42.

46. A method of altering the level of isoflavonoids in a cell of Claim 12 comprising exposing said cell to a phenylpropanoid pathway altering agent.

47. The method of Claim 46 wherein said agent is selected from the group consisting of a transcription factor and stress.

48. The method of Claim 47 wherein stress is selected from the group consisting of ultraviolet light, temperature, pressure, and phosphate level.

49. The method of Claim 47 wherein said transcription factor is a maize C1 myb-type transcription factor and a myc-type transcription factor R

50. The method of Claim 47 wherein said transcription factor is a chimera containing the maize R region between the C1 DNA binding domain and the C1 activation domain.